Report: Lipid Mixture Preparation and Dilution Protocol

1. Initial Lipid Mixture Preparation: a. In a 1 mL vial containing 600 μL of chloroform (CHCl3), add 100 μL each of MGDG, DGDG, SQDG, and DGTS (from ~1.4 mM stock solutions). b. Final concentration of each lipid: 140 μM
2. M1 Vial Preparation: a. Add 100 μL of the initial mixture to 900 μL of CHCl3. b. New concentration of each lipid in M1: 14 μM
3. MIX-1 Preparation: a. Combine in a single vial:
   * 50 μL LS-A1 (10 ng/μL)
   * 50 μL EQ101 (10 ng/μL)
   * 50 μL M1 (14 μM each lipid) b. Dry the contents completely. c. Redissolve in 50 μL solvent, labeled as MIX-1. d. Final concentrations in MIX-1:
   * LS-A1: 10 ng/μL
   * EQ101: 10 ng/μL
   * Each lipid from M1: 14 μM
4. CER-SPH-MIX-A1 Preparation: a. Sphingolipid Mix I: 25 μM of 10 compounds in ethanol (1 mL ampules) b. CER-SPH-MIX-A1: 90 μL methanol + 10 μL Sphingolipid Mix I c. Concentration of each sphingolipid in CER-SPH-MIX-A1: 2.5 μM
5. ESTD-1 Preparation: a. Take 50 μL of CER-SPH-MIX-A1, dry in vial ESTD-1. b. Add 50 μL of MIX-1 to dried ESTD-1. c. Final concentrations in ESTD-1 (50 μL total):
   * 10 sphingolipid compounds: 2.5 μM each
   * LS-A1: 10 ng/μL
   * EQ101: 10 ng/μL
   * Lipids from M1: 14 μM each
6. Initial Dilution: a. Take 10 μL from ESTD-1, add to 90 μL solvent. b. New concentrations (100 μL total):
   * Sphingolipids: 0.25 μM each
   * LS-A1: 1 ng/μL
   * EQ101: 1 ng/μL
   * Lipids from M1: 1.4 μM each
7. Serial Dilutions with 8Bu+: Perform 7 serial dilutions, each time taking 50 μL of the previous solution and adding 50 μL of 8Bu+ solvent.

Final Concentration Table:

| **Dilution** | **Sphingolipids (μM)** | **LS-A1 (ng/μL)** | **EQ101 (ng/μL)** | **Lipids from M1 (μM)** |
| --- | --- | --- | --- | --- |
| Initial | 0.25 | 1 | 1 | 1.4 |
| 1 | 0.125 | 0.5 | 0.5 | 0.7 |
| 2 | 0.0625 | 0.25 | 0.25 | 0.35 |
| 3 | 0.03125 | 0.125 | 0.125 | 0.175 |
| 4 | 0.015625 | 0.0625 | 0.0625 | 0.0875 |
| 5 | 0.0078125 | 0.03125 | 0.03125 | 0.04375 |
| 6 | 0.00390625 | 0.015625 | 0.015625 | 0.021875 |
| 7 (Final) | 0.001953125 | 0.0078125 | 0.0078125 | 0.0109375 |